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# Parasitism of the glassy-winged sharpshooter, *Homalodisca coagulata* (Homoptera: Cicadellidae): Functional response and superparasitism by *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae)☆

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**Abstract**

The functional response by the egg parasitoid, *Gonatocerus ashmeadi*, and superparasitism of *Homalodisca coagulata* eggs were found to be related to host age and density when studied under laboratory conditions. Several aspects relating to parasitism of 1-, 3-, 5-, 7-, 9-day-old *H. coagulata* eggs were measured under varied densities ranging from 1:1 to 1:60 parasitoid to host ratios. The functional response for the parasitoid to host eggs of all age groups closely fit the Type II model that describes responses to changing densities. The instantaneous attack rate and handling time of the parasitoid were similar for *H. coagulata* eggs of various ages. The number of host eggs parasitized varies significantly with host density and age, but not when analyzed by a host age × density interaction. However, host age and density, as well as the host age × density interaction, contribute significantly to the differences found in length of the development time of *G. ashmeadi* within host eggs. This parasitoid showed a significantly greater tendency toward superparasitism at parasitoid-to-host ratios ⩽10:100. The maximum number of parasitoid eggs found in a single host egg was 18. The frequencies of superparasitism for *G. ashmeadi* display a random distribution over all observed host densities. Our results also suggest *G. ashmeadi* eliminates the supernumerary parasitoids through physiological suppression.


*Keywords:* Functional response; Superparasitism; *Gonatocerus ashmeadi*; *Homalodisca coagulata*; Host age and density; Development

## 1. Introduction

The functional response defines the relationship between the numbers of hosts parasitized per parasitoid with the host or prey density over time (Holling, 1959). The analysis of functional and numerical responses of the parasitoid–host interaction is often used to determine the potential effects of parasitoids on the host population (Oaten and Murdoch, 1975). The effectiveness of a parasitoid in regulating a pest population has been traditionally related to its functional response (Hassell, 1978). Several types of functional responses have been described by models that relate to the rate of predation or parasitism on varying densities and these models may be modified by parameters such as length of exposure to the prey, attack rate, or handling time (Hassell et al., 1977). A Type I model represents a constant linear increase regardless of host density, a Type II model describes an initially constant attack rate which decelerates to a plateau as satiation is reached, a Type III model is typified by a sigmoidal rate increase, and the Type IV model describes a dome-shaped response (Luck, 1985).

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Another important attribute for successful parasitoids includes the ability to discriminate between parasitized and non-parasitized hosts (van Lenteren et al., 1978). This ability assists them avoid superparasitism and minimize the waste of time and energy associated with their searching behavior (Godfray, 1994; Mackauer, 1990). Without host discrimination, a solitary parasitoid often superparasitizes one host and causes competition between siblings through physical conflict or physiological suppression. Superparasitism by a solitary parasitoid results in the waste of its eggs, a waste of host searching and handling time, and a developmental delay often accompanied with diminished progeny size. These results usually decrease the efficiency of the parasitoids used in a biological control program. However, superparasitism is also recognized as being adaptive in certain situations (van Alphen and Visser, 1990). The advantages of superparasitism are said to increase the possibility of gaining offspring from a host and to stabilize host–parasitoid interactions in solitary and gregarious parasitoids (van Alphen, 1988; van Alphen and Visser, 1990).

*Homalodisca coagulata* (Say), the glassy-winged sharpshooter (*GWSS*) (Homoptera: Cicadellidae), has become a great threat to many agricultural and ornamental crops in California of the United States of America because it vectors the xylem-inhabiting bacterium, *Xylella fastidosa* Wells. This bacterium causes Pierce's disease in grapes and similar diseases in numerous other crops (Blua et al., 1999). Recently, *H. coagulata* has become the focus of a major classic biological control program in California (Irvin and Hoddle, 2005). Egg parasitoids in the families Mymaridae and Trichogrammatidae have been released across nine California counties (CDFA, 2003). Among its natural enemies, *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae) is an important species that accounts for 80–95% of the observed parasitism on the sharpshooter eggs in California (Phillips, 2000). In Florida, *G. ashmeadi* is considered as one of the extremely efficient parasitoids of the eggs of *H. coagulata* (López et al., 2004). Previous studies of *G. ashmeadi* have focused on parasitism (Irvin and Hoddle, 2005), overwintering biology (López et al., 2004), mymarid taxonomy (Triapitsyn, 2003; Triapitsyn et al., 1998), and field release investigations (Phillips, 2000). No studies have been conducted to establish the relationship between host densities and rates of attack for *G. ashmeadi*. Moreover, the rates of development and superparasitism of this parasitoid are not known.

The objectives in this study are as follows: (1) to determine the type of functional response of this parasitoid as related to *H. coagulata* eggs of different ages; (2) to assess the frequency of superparasitism in an effort to evaluate whether this parasitoid deposits its eggs in a random or non-random fashion; and (3) to determine the effect of host density and age, and superparasitism with respect to the development and emergence of the parasitoid. It is expected that this information will be useful in assessing the efficiency of *G. ashmeadi* as a biological control agent of *H. coagulata*, devising mass-rearing protocols, and implementing release programs for this parasitoid.

## 2. Materials and methods

### 2.1. GWSS colonies

The *H. coagulata* colonies used in this study were originally derived from *GWSS* colonies maintained at the USDA/APHIS Plant Protection Laboratory, Edinburg, TX. All the nymphs were reared in Plexiglass cages ($40 \times 40 \times 60$ cm) on sunflower plants (*Helianthus annuus* L.) in an environmental chamber (25 °C, RH 60%, and 14 L:10D). Fresh plants were introduced each week during the nymphal stages. Upon adult emergence, 70–100 adults were placed into tent-like cages (Bug Dorm-2, BioQuip Products) containing a mixed host system consisting of sunflower (*H. annuus*), an evergreen shrub (*Euonymus japonica* Thunb.), and chrysanthemum (*Chrysanthemum morifolium* L. va. 'White Diamond') plants in a greenhouse augmented with sodium lighting having a photoperiod of 16L:8D. These cultivars were cultured in black plastic pots (11.5 cm diameter × 10 cm high) containing Sunshine soils (Sun Gro Horticulture Canada). The plants were watered daily, and fertilized weekly by using 5% liquid Prolific$^{TM}$ 20-20-20 (Terra International). After 7 or 8 generations, the *GWSS* eggs collected from the colonies in greenhouse were used for the tests.

### 2.2. Parasitoid rearing

Our *G. ashmeadi* colony originated from a colony maintained at the California Department of Food and Agriculture, Mt. Rubidoux Field Station, Riverside, CA. The parasitoids for these studies were maintained at $22 \pm 1$ °C on a photoperiod of 10 L:14 D in the plastic tent-cages in the laboratory. Pots of chrysanthemum plants bearing *H. coagulata* egg masses were exposed to the caged *G. ashmeadi* colonies every 15–16 days to maintain a steady supply of parasitoids. Before each test, the euonymus plants bearing 1–3-day-old *GWSS* eggs were exposed to the parasitoid colonies for 24 h to collect parasitized eggs. Upon emergence, the newly emerged wasps were collected every morning and afternoon, and individually put into clean cages. The procedure ensured that all wasps tested were of the same age. These wasps were fed with honey and water, and then at the age of 2 days postemergence, the wasps were used in the tests.

### 2.3. Functional response studies

The treatment protocols included five host ages (1, 3, 5, 7, and 9 days) and targeted host densities of 10, 20, 30, 40, 50, and 60 *GWSS* eggs per parasitoid female. Embryonic age was determined according to the developmental time of the insects at a constant temperature of $22 \pm 1$ °C. The petiole of the excised euonymus leaves bearing *GWSS* eggs

of different ages was placed into a moistened sponge in a petri dish (3.5 cm diameter × 1.0 cm high) to prevent egg mass desiccation within a transparent container (26 cm diameter × 9 cm high, Tristateplastic, USA). The transparent containers with the lids covered with fine nylon mesh, were placed upside down before putting in the petri dish. Because about 94% egg masses had brochosomes deposited by *GWSS* females during oviposition (Hix, 2001), the amounts varied among the egg masses, and the brochosomes evidently slow down the time that parasitoids spend in completing their oviposition (Jones, 2002), we felt it necessary to normalize our samples by removing any brochosomes present on the hosts. Therefore, the white powdery brochosomes were gently washed away using tap water before test. Since the number of eggs per egg mass was variable, construction of the host densities used in this study consisted of one or more than one egg mass located on several leaves. Each of host densities had same number of host leaves to avoid a possible affect of spatial heterogeneity on parasitoid behavior. One parasitoid was introduced into each container. All experiments were conducted at room temperature (22 ± 1 °C) and a photoperiod of 10L:14D, with lighting provided by fluorescent lights. After 24 h, wasps in each container were removed and their survival was recorded. If a female died during experiment, data from that container were not used. After the removal of the parasitoids, the containers were placed at 22 ± 1 °C to allow parasitoids to develop into adults and emerge from the *GWSS* eggs. The following parameters were recorded: (1) the developmental time of parasitoids within host eggs; (2) number of emerged wasps; (3) number of wasps dying within host eggs during development. The number of eggs parasitized but containing dead or undeveloped parasitoids was determined by dissecting each host egg mass under a stereomicroscope 3 days after wasp emergence ceased. Therefore, the total number of *GWSS* eggs parasitized = the number of emerged wasps + the number of eggs containing wasps that died during development. These experiments were replicated 10 times.

### 2.4. Superparasitism studies

The female parasitoid/host ratios tested in this experiment were 1:1, 1:5, 1:10, 1:15, 1:20, and 1:25. A control ratio of 0:100 was also used to assess any naturally occurring host mortality. The actual number of parasitoids per host egg per container was 10:10, 10:50, 10:100, 10:150, 10:200, 10:250, and 0:100. Host egg masses were prepared as described above before exposure to parasitoids. Upon emergence, female and male parasitoids were caged and provided with honey and water. A potted euonymus plant without host eggs was placed into the cage to acclimate parasitoids to the host plant before the experiments were initiated.

After the 2- to 3-day-old host eggs were exposed to parasitoids for 48 h, they were removed. In every treatment, except for the experiment using a 1:1 ratio, 20 host eggs were randomly selected, dissected, and the number of developing wasps were recorded. All host eggs in the experiment using a 1:1 ratio were dissected. To determine whether there was an effect of superparasitism on length of development of *G. ashmeadi*, the rest of parasitized host eggs were placed in plastic containers and held in the laboratory to record parasitoid eclosion. These experiments were replicated 5 times.

Dissections were performed in an alcoholic solution of 0.02% eosin under a stereomicroscope. The leaf tissue covering the egg masses was carefully removed by using a pair of fine forceps. Sharpshooter eggs were dissected by grasping the anterior end with a pair of fine forceps and by removing the posterior end with a sharp scalpel. The egg contents were gently squeezed into the dissecting solution. The parasitoid eggs and wasp larvae remained unstained while the tissues of sharpshooter egg were stained red with the eosin. Numbers of parasitoid eggs and larvae present in each sharpshooter egg were recorded.

### 2.5. Statistical analysis

All statistical analyses were performed using SAS System for Windows (SAS Institute Inc., 1996) at a significance level of $\alpha = 0.05$. The data from the functional response studies were analyzed using two-way ANOVA to test if both ages and egg densities affected the number of eggs parasitized and development of parasitoid within the host egg.

Parasitoid functional response data for each experimental shape and host age were analyzed in two steps (Juliano, 2001). In the first step, the shape of the functional response curve was determined by logistic regression analysis of the proportion of *H. coagulata* eggs parasitized as a function of initial density (Trexler et al., 1988). In the second step, the random predator equation was fitted to data after the functional response type was determined (Juliano, 2001) because prey depletion without replenishment was the design in our experiments.

A polynomial function (Juliano, 2001) was used to fit the data on the proportion of host eggs parasitized:

$$\frac{N_e}{N_0} = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}{1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}, \quad (1)$$

where $N_e$ is the number of host eggs parasitized, $N_0$ is the number of initial host, $N_e/N_0$ is the probability of being parasitized, and $P_0$, $P_1$, $P_2$, and $P_3$ are the parameters to be estimated. These parameters can be estimated by using the CATMOD procedure in SAS software (Juliano, 2001). The signs of the linear ($P_1$), quadratic ($P_2$) parameters from Eq. (1) can be used to distinguish the shape of the functional response from experimental data. Linear terms not significantly different from 0 indicate a Type I functional response. A positive linear parameter ($P_1$) and a negative quadratic parameter ($P_2$) indicate that functional response is Type III, whereas if both parameters are negative, the

functional response is Type II (Juliano, 2001; Trexler et al., 1988).

Once the functional response type was determined from logistic regression analyses, the non-linear least squares regression was used to fit the Holling's disc equation (Holling, 1959) to data because Rogers' random equation (Rogers, 1972) produced invalid parameters($T_h$ was negative for 3-, 5-, 7- and 9-day-old host eggs) (NLIN procedure, SAS). The Holling's disc equation was as follows:

$$N_e = aTN_0/(1 + aT_hN_0), \quad (2)$$

where $N_e$ is the number of parasitized host; $N_0$, the number of host offered; $T$, the total time available for the parasitoid; $a$ is the attack rate, and $T_h$, the handling time. Comparison of parameter ($a$ and $T_h$) between every two host ages was performed by using Eq. (3) with indicator variables (Allahyari et al., 2004):

$$N_e = [a + D_a(j)]N_0^2T/\{1 + [T_h + D_{T_h}(j)]N_0^2[a + D_a(j)]\} \quad (3)$$

where $j$ is an indicator variable that takes value 0 for certain host age, and 1 for another host age. The parameters $D_a$ and $D_{Th}$ estimate the differences between the host age in the value of the parameters $a$ and $T_h$, respectively. Separation of statistically different parameter estimates was made using 95% confidence intervals in the NLIN procedure (SAS). Parameter estimates were not significantly different if comparisons produced 95% confidence intervals that included zero (Juliano, 2001).

In the superparasitism experiments, separate analysis of variance (ANOVA) and Duncan's multiple mean comparison were used to analyze the differences in the number of parasitoid eggs, percentage of adult eclosion, and developmental time of parasitoids within host eggs for the different parasitoid/host ratios. A square-root transformation was applied to the values representing percentage adult eclosion before analysis to normalize the differences in the observed samples. The relationships between the number of parasitoid eggs per host egg and parasitoids to host egg ratios, between the number of enclosed adults and the number of parasitoid eggs per host egg, were analyzed by simple linear regression (PROC LIN). Data dealing with parasitoid eggs per host egg were further analyzed by computing the expected Poisson distribution of sharpshooter eggs containing 0, 1, 2, 3, and >3 parasitoid eggs (van Lenteren et al., 1978). Based on the data pooled for similar ratios of parasitoids to host eggs, a total of 30 ranges were defined for *G. ashmeadi*. A $\chi^2$ goodness-of-fit test was performed (PROC FREQ) to determine whether pooled parasitoid egg distributions were significantly different from expected Poisson distributions. The variance and mean (PROC MEANS) for the number of parasitoid eggs per host eggs were calculated through analyses of superparasitism data pooled from similar parasitoid/host ratios as previously described.

Taylor's Power Law (Taylor, 1961) was used to describe the relationship between the variances ($S^2$) and means ($m$): $S^2 = am^b$, where $a$ and $b$, where $a$ is largely a scaling factor related to sample size, and $b$ appears to be an "index of aggregation characteristic of the species". That is, when $b > 1$, there is an aggregated distribution; when $b = 1$, there is a random distribution; and when $b < 1$, there is a regular distribution. For this output, $t$ tests were conducted for the null hypothesis ($H_0$: $\mu = 1$, $t = [\text{slope} - 1]/\text{SE}$ of slope, degrees freedom = N − 1) to determine if there was significant difference between $b$ value and 1. This analysis allowed us to test whether random, regular or aggregated superparasitism distribution was representative of *G. ashmeadi* over the different host densities.

## 3. Results

### 3.1. Microscopic observations of the parasitoid

The elongate, fusiform egg of *G. ashmeadi* ranges from 220 to 250 μm in length (Fig. 1A). Hatching occurs at 24–48 h postoviposition and at this age the 1st instar wasp is also fusiform in shape, has hook-shaped mandibles, a tail and readily displays mobility upon separation from the host (Fig. 1A). Approaching transition of the 1st to the 2nd instar at 4–5 days postoviposition, the larvae increase in length to about 1 mm (including tail) and have a more robust appearance (Fig. 1B).

In superparasitized egg hosts at 96 h after exposure to the wasps, we have found the 2nd instars and occasionally unhatched parasitoid eggs along with live and moribund 1st instars (Fig. 1C). While the supernumerary1st instars are either dead or dying, they do not show obvious signs of having been attacked by their counterparts sharing the same host. The 2nd and 3rd instars are without tails, rounded on both the anterior and posterior ends, have no noticeable head structure, and about 0.7–0.75 mm in length (Figs. 1D and E). Under conditions of superparasitism there may be two 2nd instars or one 2nd and one 3rd instar found in a single host egg, but typically one larva is noticeably larger than its counterpart(s) (Fig 1D).

The 3rd instar parasitoids are evident in *GWSS* egg hosts exposed to wasps 7 days previously. At this stage the larvae are about 1 mm in length and, unlike the earlier larval stages, the mouthparts do not readily take up the eosin stain and are thus not easily discernable (Fig. 1E). The 2nd and 3rd instar wasps do not show signs of mobility when dissected from the host.

Figs. 1F and G show early and late stage pharate adults that were photographed upon dissection from the host 10 days after exposure of the wasps to the sharpshooter eggs. Fig. 1F displays an intact pupa and Fig. 1G shows a pharate adult dissected from the host shortly before emergence.

### 3.2. Parasitoid efficiency and development time

Table 1 shows the efficiency of parasitism and development time by *G. ashmeadi* when offered different den-

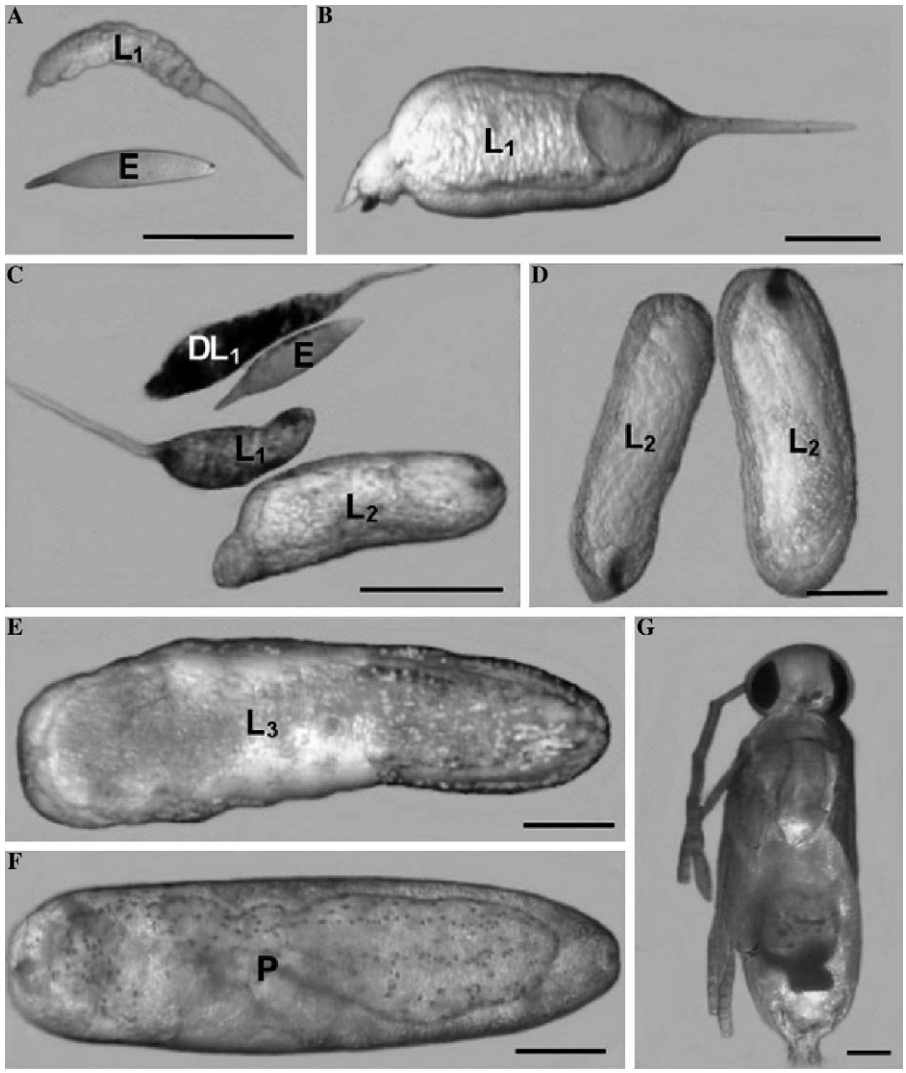


Fig. 1. (A–F). Developmental stages of *G. ashmeadi* within *H. coagulata* eggs. (A) parasitoid egg and early 1st instar larva 2 days postoviposition; (B) late 1st instar larva 3 days postoviposition; (C) 2nd instar larvae 4–5 days after oviposition; (D) 3rd instar larva 7 days postoviposition; (E) late stage pupa (pharate adult) 10 days after oviposition; (F) adult before emergence. E = egg, $L_1$ = 1st instar larva, $DL_1$ = degenerating larva, $L_2$ = 2nd instar, L3 = 3rd instar, *P* = pupa. Bar = 300 μm.

sities of the *H. coagulata* egg host over a 24 h observation period. At a ratio of 1 wasp to 10 eggs, about 9 out of every 10 eggs were successfully parasitized for all ages of the host eggs that were tested. However, except for the 1- and 3-day-old eggs, the mean per cent efficiency of parasitism had the greatest decline at the level of 1 wasp to 20 eggs for the 5-, 7-, and 9-day-old hosts (Fig. 2). At a density of 60 eggs to 1 wasp, the range of parasitism was 50–55%, meaning that about half the egg hosts produced wasp progeny regardless of age. The number of 1-day-old *GWSS* eggs parasitized by *G. ashmeadi* was greater than that of 5-, 7-, and 9-day-old eggs parasitized. A two-way ANOVA, with age, density as factors, revealed that the number of eggs parasitized varied significantly with host age ($F_{4,299} = 3.64$, $P = 0.007$) as well as host densities ($F_{5,299} = 88.43$, $P < 0.0001$). There was no significant effect of the age × density interaction on the number of host eggs parasitized ($F_{20,299} = 0.44$, $P = 0.899$).

The development time of *G. ashmeadi* within host eggs varied significantly with host density and host age. Within the host eggs parasitized at 1-, 3-, 5-, 7- and 9-day-old, the mean development time of the parasitoids in days was 16.0 ($n = 1435$), 18.9 ($n = 996$), 18.3 ($n = 1181$), 17.6 ($n = 961$), and 17.8 ($n = 1254$), respectively. Thus, the parasitoids starting development within 1-day-old sharpshooter eggs developed significantly faster than the other ages ($F_{4,5826} = 766.41$, $P < 0.0001$). Two-way ANOVA further showed that host age ($F_{4,5826} = 999.47$, $P < 0.0001$) and density ($F_{5,5826} = 58.26$, $P < 0.0001$) contributed significantly to the development time of *G. ashmeadi*. The significant interactive effect on development time occurred between host age and density ($F_{20,5826} = 62.82$, $P < 0.0001$).

Table 1
Parasitism efficiency and egg to adult development time of *G. ashmeadi* as a function of *H. coagulata* age and density

| Wasp:egg ratio | Age of host | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 day | | 3 days | | 5 days | | 7 days | | 9 days | |
| | No. parasitized | Development time (days) | No. parasitized | Development time (days) | No. parasitized | Development time (days) | No. parasitized | Development time (days) | No. parasitized | Development time (days) |
| 1:10 | 9.5 ± 1.3 a | 15.9 ± 0.6 d | 8.7 ± 2.2 a | 21.0 ± 2.1 a | 8.9 ± 1.6 a | 17.9 ± 1.6 c | 9.0 ± 2.2 a | 15.7 ± 1.4 e | 9.1 ± 1.1 a | 17.6 ± 1.4 c |
| 1:20 | 18.1 ± 1.6 b | 16.5 ± 0.8 a | 15.5 ± 3.2 b | 18.5 ± 1.6 c | 14.8 ± 3.4 b | 18.0 ± 1.1 c | 14.6 ± 3.9 ab | 18.3 ± 0.9 a | 14.7 ± 3.3 ab | 18.3 ± 0.8 b |
| 1:30 | 22.9 ± 3.0 c | 16.5 ± 0.7 b | 17.9 ± 8.4 b | 18.6 ± 2.2 c | 22.0 ± 5.8 c | 18.8 ± 1.3 b | 19.8 ± 7.7 bc | 18.1 ± 0.9 ab | 18.7 ± 4.1 b | 19.1 ± 1.2 a |
| 1:40 | 26.5 ± 4.7 cd | 16.1 ± 0.8 c | 22.2 ± 9.8 bc | 18.3 ± 2.2 c | 25.1 ± 7.3 cd | 17.8 ± 1.5 c | 22.7 ± 5.6 c | 18.0 ± 1.0 bc | 25.8 ± 6.2 c | 16.9 ± 1.6 d |
| 1:50 | 30.3 ± 7.5 d | 16.0 ± 1.4 cd | 25.6 ± 10.0 cd | 18.6 ± 1.5 c | 29.4 ± 5.1 de | 19.5 ± 1.2 a | 23.9 ± 11.9cd | 17.8 ± 0.6 c | 29.5 ± 13.1 c | 17.4 ± 1.4 c |
| 1:60 | 34.8 ± 4.7 e | 15.5 ± 0.7 e | 30.7 ± 6.9 d | 19.4 ± 1.0 b | 32.2 ± 4.5 e | 17.4 ± 1.0 d | 29.9 ± 7.3 d | 17.2 ± 1.2 d | 30.1 ± 3.4 c | 18.1 ± 1.2 b |
| | $F = 43.12$ | $F = 45.39$ | $F = 11.02$ | $F = 37.00$ | $F = 31.69$ | $F = 88.13$ | $F = 10.59$ | $F = 84.08$ | $F = 16.96$ | $F = 73.93$ |
| | $df = 5.59$ | $df = 5,1434$ | $df = 5.59$ | $df = 5,995$ | $df = 5.59$ | $df = 5, 1180$ | $df = 5.59$ | $df = 5,960$ | $df = 5.59$ | $df = 5,1253$ |
| | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ | $P < 0.001$ | $P < 0.0001$ |

Means ± SE within columns followed by different letters are significantly different at the 0.05 level (SAS PROC GLM) in ANOVA (Duncan).

### 3.3. Functional responses

The functional responses of *G. ashmeadi* to host eggs of various ages and densities are displayed in Fig. 2. Logistic regression analyses of the proportion of host eggs parasitized (Table 2) indicated that the functional response of the parasitoid to host eggs of different ages was that of a Type II model. However, estimates of the linear coefficient for 1-, 5- and 7-day-old host eggs were not significantly different from 0 ($P > 0.05$), and only the estimates of the quadratic coefficient for 9-day-old host eggs were significantly different from 0 ($P = 0.0003$), suggesting that the data for host eggs of age of 1–7 days should be interpreted with caution. The instantaneous attack rates ($a$) for 1-, 3-, 5-, 7- and 9-day-old host eggs estimated by Holling's disc equation were 0.58, 0.45, 0.50, 0.51, and 0.48 day$^{-1}$, and handling times ($T_h$) were 0.03,0.03, 0.03,0.04 and 0.03 day, respectively (Table 3). Comparison of instantaneous attack rate and handling time between every two host ages showed that asymptotic 95% confidence intervals for $a$, $D_a$, $Th$ and $D_{T_h}$ included 0, suggesting that there were no significant differences in attack rate and handling time among host ages (Table3).

### 3.4. Superparasitism

The level of superparasitism of *G. ashmeadi* (Table 4) varied significantly with increasing host density ($df = 5,549$, $F = 225.17$, $P < 0.0001$). The mean number of parasitoid eggs recorded per sharpshooter egg at a 10:10 parasitoid/host ratio is significantly greater than that at other parasitoid/host ratios and, in some cases, reaches astonishing levels. For example, the maximum number of parasitoid eggs dissected from one host egg in the 10:10 treatment group was 18. When the ratio of parasitoids to hosts increased to $\geqslant$10:150, host eggs pooled from each host density were nearly all parasitized. There was a significant positive correlation between the number of parasitoid eggs per host egg and parasitoid/host ratio ($F = 1231.69$, $df = 548$, $r^2 = 0.692$, $P < 0.0001$). *G. ashmeadi* is typically a solitary parasitoid, with only one wasp emerging from each egg of the host. In treatments with a high density of parasitoids such as the 10:100 and 10:50 parasitoid/host ratios, the percentage of parasitoid eclosion per egg was significantly higher than in the treatments having a low density of wasps ($F = 3.996$, $df = 4,243$, $P = 0.004$)(Table 4). Although there was significant difference in development time of the parasitoids within hosts among the different parasitoid/host ratios ($F = 46.851$, $df = 4,1862$, $P < 0.0001$), the maximum difference only was about 0.7 day.

The distribution of parasitoid eggs (Fig. 3) showed the percentage of host eggs superparasitized (parasitoid eggs/host egg were equal to or greater than 2) decreased significantly with the parasitoid to host ratios ($F = 29.52$, $df = 5,29$, $P < 0.001$). When the ratio was 10/10 and 10/50, the percentage of superparasitism was 100% and about

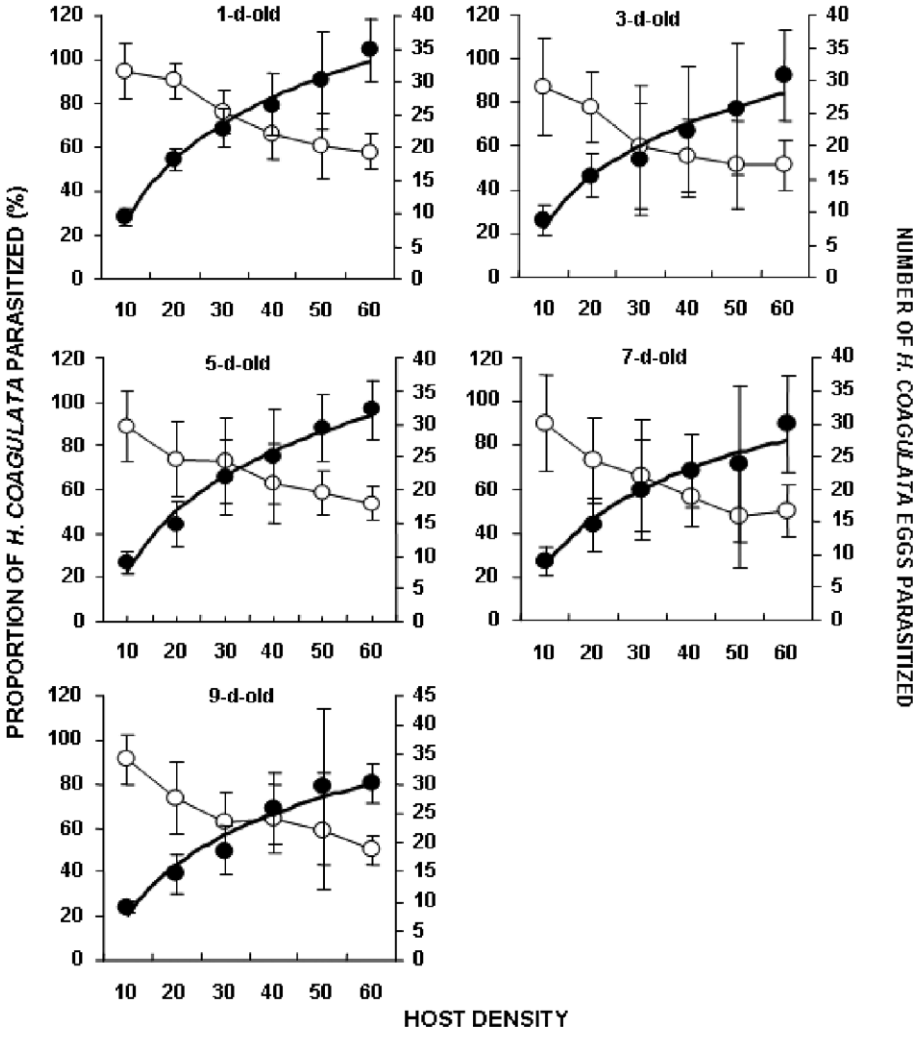


Fig. 2. Functional responses of *G. ashmeadi* to *H. coagulata* eggs of 1, 3, 5, 7, and 9 days of age. Observed numbers (filled circles) and the proportions (open circles) of host eggs parasitized are indicative of the means ± SE.

Table 2
Results of logistic regression analyses of the proportion of *H. coagulata* eggs parasitized by *G. ashmeadi* compared to initial host numbers offered

| Host age | Parameter | Estimate | SE | $\chi^2$ | Probability |
|---|---|---|---|---|---|
| 1-day-old | Constant | 4.7734 | 1.1394 | 17.55 | <0.0001 |
| | Linear | −0.1754 | 0.0968 | 3.28 | 0.0696 |
| | Quadratic | 0.00212 | 0.00227 | 0.68 | 0.4096 |
| | Cubic | −0.0000072 | 0.0000021 | 0.11 | 0.7369 |
| 3-day-old | Constant | 3.3455 | 0.7606 | 19.35 | <0.0001 |
| | Linear | −0.1673 | 0.0695 | 5.79 | 0.0161 |
| | Quadratic | 0.00280 | 0.00195 | 2.07 | 0.1503 |
| | Cubic | −0.00002 | 0.000017 | 0.84 | 0.3607 |
| 5-day-old | Constant | 2.6881 | 0.7580 | 12.58 | 0.0004 |
| | Linear | −0.0943 | 0.0701 | 1.81 | 0.1786 |
| | Quadratic | 0.00136 | 0.000197 | 0.47 | 0.4916 |
| | Cubic | −0.0000080 | 0.0000017 | 0.22 | 0.6383 |
| 7-day-old | Constant | 2.8370 | 0.7639 | 13.79 | 0.0002 |
| | Linear | −0.0968 | 0.0699 | 1.91 | 0.1664 |
| | Quadratic | 0.000678 | 0.000196 | 0.12 | 0.7293 |
| | Cubic | 0.0000023 | 0.0000017 | 0.02 | 0.8909 |
| 9-day-old | Constant | 4.5942 | 0.8313 | 30.54 | <0.0001 |
| | Linear | −0.3040 | 0.0747 | 16.55 | <0.0001 |
| | Quadratic | 0.00753 | 0.00207 | 13.27 | 0.0003 |
| | Cubic | −0.00006 | 0.000018 | 12.28 | 0.0004 |

Table 3
Attack rate and handling time (means ± SE) of the functional response of *G. ashmeadi* females to densities of *H. coagulata* eggs of different ages[a]

| Host age (day) | $a$ (day$^{-1}$) | $T_h$ (day) | $r^2$ |
|---|---|---|---|
| 1 | 0.5782 ± 0.0626 a | 0.0300 ± 0.0004 a | 0.97 |
| 3 | 0.4544 ± 0.0959 a | 0.0315 ± 0.0105 a | 0.90 |
| 5 | 0.5013 ± 0.0640 a | 0.0286 ± 0.0058 a | 0.96 |
| 7 | 0.5064 ± 0.0188 a | 0.0377 ± 0.0099 a | 0.90 |
| 9 | 0.4831 ± 0.0849 a | 0.0296 ± 0.0082 a | 0.93 |

[a] Parameters in Type II model were compared using an equation with indicator variables (0 and 1) (see Juliano, 2001). Means followed by the same letters within columns are not significantly different (comparison of 95% confidence intervals).

85%, respectively, significantly greater than that for other ratios (10:100 = 67%; 10:150 = 51%; 10:200 = 25%; and 10:250 = 25%). Fittingly, the percentage of host eggs containing only one parasitoid egg per host varied significantly with the parasitoid to host ratios ($F = 20.17$, $df = 5,29$, $P < 0.001$). As the number of parasitoids to hosts became lower, there was a consequent increase in the percentage

Table 4
Number (means ± SE) of *G. ashmeadi* eggs observed within each host egg, percentage emergence, and development time at different parasitoid/host egg ratios[a]

| Parasitoid:host ratio | No. parasitoid/host | | % Emergence | | Development time | |
|---|---|---|---|---|---|---|
| | $N_1$ | Means ± SE[a] | $N_2$ | Means ± SE[a] | $N_3$ | Means ± SE[a] |
| 10:10 | 50 | 10.40 ± 4.86 a | [b] | [b] | [b] | [b] |
| 10:50 | 100 | 3.02 ± 1.69 b | 11 | 97.6 ± 1.7 a | 141 | 18.02 ± 0.07 a |
| 10:100 | 100 | 2.24 ± 1.16 c | 15 | 98.9 ± 0.6 a | 136 | 18.20 ± 0.06 b |
| 10:150 | 100 | 1.66 ± 0.89 d | 77 | 93.6 ± 1.5 b | 490 | 18.30 ± 0.04 b |
| 10:200 | 100 | 1.20 ± 0.59 d | 70 | 91.7 ± 1.0 b | 263 | 18.25 ± 0.04 b |
| 10:250 | 100 | 1.15 ± 0.58 d | 71 | 90.0 ± 1.7 b | 833 | 18.77 ± 0.03 c |

[a] Means within columns followed by different letters are significantly different ($P < 0.05$, GLM) in ANOVA (Duncan). $N_1$ represents the number of dissected host eggs, $N_2$ represents the number of egg masses observed, and $N_3$ equals the number of parasitoids emerging from host eggs.
[b] Data on percentage emergence and developmental time were not available because all parasitized host eggs at the level of 1 parasitoid to 1 host egg were dissected.

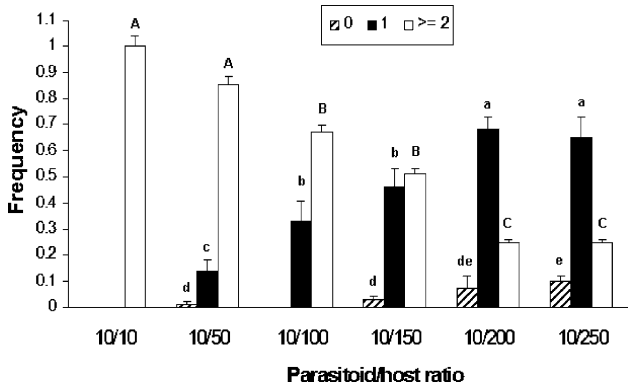


Fig. 3. Distribution of the number of parasitic eggs/host at different parasitoid to host ratios. The number of parasitic eggs recorded per host was divided into three categories (0, 1, and ⩾2) and the distribution of these categories compared to the various parasitoid to host ratios. Bars followed by different letters are significantly different at the level of 0.05 (ANOVA with LSD).

of hosts containing only one parasitoid. When the ratio was 10/200 and 10/250, the percentage of hosts containing one parasitoid was 68 and 65%, respectively. This was significantly higher than that for other ratios (10:50 = 14%; 10:100 = 33%; and 10:150 = 46%) (Fig. 3). We also found that 1, 3, 7, and 10% of host eggs were not parasitized at all when the parasitoid to host ratio was 10:50, 10:150, 10:200, and 10:250, respectively. Thus, there was significant difference among the percentages in unparasitized eggs ($F = 3.87$, $df = 5,29$, $P < 0.01$).

For *G. ashmeadi*, $\chi^2$ goodness-of-fit analyses of parasitoid egg frequency per host egg revealed that frequencies of superparasitism were significantly different from the expected Poisson distribution over all host densities ($\chi^2 = 231.291$, $df = 4$, $P < 0.0001$). The relationship between the variances ($S^2$) and means ($m$) was described as: $\log S^2 = -0.4384 + 1.0288 \log m (r^2 = 0.604$, $df = 28$, $F = 42.78$, $P < 0.0001$), where $b = 1.0288 > 1$. However, $b$ value was not significantly greater than 1 ($t = 0.18$, $df = 28$, $P > 0.05$). This indicates that there is a random distribution of superparasitism for *G. ashmeandi* over all experimental parasitoid to host ratios used in this study.

## 4. Discussion

### 4.1. Functional response

The type of functional response of *G. ashmeadi* when exposed to *H. coagulata* eggs was not influenced by the age of the host. The parasitoid shows a similar Type II functional response to 1- through 9-day-old host eggs. The Type II model is a commonly observed model for parasitoids, especially when parasitoids are caged with their hosts in a small space for a fixed period of time (van Lenteren et al., 1978).

The parameters of a functional response as estimated by Holling's disc equation show that the instantaneous attack rates ($a$) and handling time ($T_h$) are not affected by host ages during the experimental periods used in this study (Table 3). These results indicate that the parasitoid has no age-preference when caged with their hosts in a small container for 24 h. Leopold et al. (2003) also reported that *G. ashmeadi* shows no host age-preference for *H. coagulata* eggs from 0 to 5 days of age when exposed to high density of parasitoids under no choice conditions. However, when the exposure time is just for 2 h, the age of *H. coagulata* eggs significantly influences the incidence of parasitism by *G. ashmeadi* under no choice conditions (Irvin and Hoddle, 2005). In this study, using eggs 3 days of age resulted in the highest levels of parasitism. When using undetermined parasitoid to egg ratios, López et al. (2004) reported that the parasitism rate of *G. ashmeadi* declined on 7- through 9-day-old eggs of the *GWSS* on excised holly leaves but not for the egg masses on leaves left on the plant.

Handling time is a general term that includes time for parasitoid searching, antennating, probing, and parasitizing a host, and also the time spent resting and preening. The handling times for *G. ashmeadi* as estimated from Type II functional response curves are similar (Table 3). In other words, this parasitoid spends about the same amount of time handling the hosts, regardless of host age. These results contrast with the investigation made by van Huis et al. (1991) on *Uscana lariophaga* Steffan, an egg parasitoid of several bruchid species, which showed that time

required for probing, i.e., penetrating the egg chorion, increases with host age.

Handling time is one of the most important characters of the host–parasitoid interaction. The handling time of *G. ashmeadi* calculated on the 24 h observation period used in this study is 0.03–0.04 day (Table 3). When converted to minutes this time is about 43–58 min. Since the experiments were run under a 10L:14D photoperiod, it stands to reason that the activity of the wasps is limited during the dark phase of the observation period and that the handling time is overestimated. Our laboratory observations suggest that these wasps spend about 20–25 min per egg (data not shown). Theoretically, the ratio between exposure time and handling time ($T/T_h$) is an indicator of maximum parasitism. In our experiment, the maximum number of host eggs parasitized by *G. ashmeadi* per day is 34.6, 30.7, 32.2, 29.9, and 30.1 for 1-, 3-, 5-, 7-, and 9-day-old *H. coagulata* eggs under the photoperiod of 10L:14D.

The similarity in the instantaneous attack rate ($a$) indicates that *G. ashmeadi* females have the same parasitization activity regardless of the age of *H. coagulata* eggs. We have observed the ovipositional process of *G. ashmeadi*, which is divided into several steps as follows: (1) landing on a plant bearing a host egg mass, antennating leaf surfaces and searching for an egg mass; (2) cessation of antennation upon finding egg mass and orienting for oviposition; (3) making multiple ovipositions within the egg mass; (4) leaving the egg batch, moving about the leaf and then stopping to groom; (5) starting the process over with the step # 1. Because superparasitism occurs at low host densities (discussed below), a female may lay eggs in already parasitized eggs when returning to the egg batch. However, superparasitized eggs are statistically considered to have only one parasitoid egg when experimental data are collected after adult emergence because solitary parasitoids typically produce only one progeny per host regardless of the number of wasp ovipositions that have occurred. Thus, when fitted with functional response model, the data may result in underestimation of attack rate and an overlap of handling time.

While *G. ashmeadi* successfully completes the development within host eggs regardless of age, the developmental time is significantly affected by the age of its host. The parasitoid develops faster within 1-day-old host eggs than other ages (Table 1). In the older *H. coagulata* eggs, the advanced embryogensis may inhibit *G. ashmeadi* development (Irvin and Hoddle, 2005). Host eggs are expected to provide fewer resources with increasing age because host nutrients have been transformed into substances that presumably can not be readily assimilated by developing parasitoids (Vinson, 1990). Thus, physiological changes occurring with advancement of host age results in a developmental slow down of the parasitoid.

### 4.2. Superparasitism

Our results show that the frequency of superparasitism by *G. ashmeadi* decreases with an increase in host density and is accompanied with an increase in unparasitized eggs (Fig. 3). This suggests that the limitation of the host resources induces wasps to superparasitize, as was observed with *G. ashmeadi*. Previous studies have also shown that parasitoids adaptively superparasitize when perceiving a limitation of host resources (van Alphen and Visser, 1990; Weisser and Houston, 1993).

The superparasitism exhibited by *G. ashmeadi* suggests that this solitary egg parasitoid lacks the ability to discriminate between unparasitized and conspecifically parasitized hosts. This type of behavior also has been documented in other parasitoids (Gardner et al., 1984; Heitland and Pshorn-Walcher, 1992). Fig. 3 also shows that there still are some host eggs that are not parasitized, even when parasitoid to host ratio is 10:50. This lack of the host discrimination ability further accounts for the behavior of *G. ashmeadi* as observed in this study.

Despite the existence of superparasitism, rarely does more than one *G. ashmeadi* emerge from a host egg. In solitary species, the elimination of supernumerary parasitoids in host egg can be caused by cannibalism and/or physiological suppression (Mackauer, 1990). In a number of parasitic wasp species, the 1st instar mobile larvae are apparently adapted for aggressive conflict with their siblings. The competition for host resources has been reported to result in decrease in emergence and development arrest (Danyk et al., 2000). However, our experiments show that the decrease in overall parasitoid emergence (Table 4) is caused by high rate of unparasitized eggs (Fig. 3). Further, the dominant parasitoid larva in the hosts initially having $\geqslant 3.02$ eggs/host develops slightly faster than that in hosts having $<2$ eggs/host. These results show that elimination of supernumerary conspecific larvae is without obvious costs in terms of the development of the dominant parasitoid. In addition, except for the 1st instar larvae, the 2nd and 3rd instars display little, if any, mobility. Thus, the probability of supernumerary parasitoids in host eggs are eliminated at this stage through physical combat is not supported by our observations. All observations suggest that physiological suppression may be the mechanism to eliminate of supernumerary parasitoids. The more dominant larva may make the host environment unsuitable for the younger cohorts to continue their development by production of proteolytic enzymes (Mellini, 1990) or reduction of nutrients by degradation of the host tissues.

Nevertheless, superparasitism is generally detrimental to solitary parasitoids because it represents a waste of the overall reproductive potential of the parasitoid. From the applied point of view, superparasitism by *G. ashmeadi* could result in a decrease in the efficiency of a biological control program because: (1) superparasitism represents a waste of the production colony's potential output by wasting eggs and searching time (Sirot and Krivan, 1997) and; (2) superparasitism can cause a decrease in the overall parasitism rate of pest populations when using an inundative release for control purposes (van Dijken and van Alphen, 1991).

In conclusion, the natural enemies (parasitoids or predators), pests, habitat, and environmental conditions are four main factors of an interactive ecosystem that need to be understood to conduct a successful IPM program (Duffey et al., 1986; Parajulee et al., 1994). Our results emphasize the impact of host age on functional response types and superparasitism by *G. ashmeadi*. They suggest that the parasitoid could perform as an efficient biological control agent of all ages of *H. coagulata* eggs found in an agricultural setting. The baseline information obtained from observing laboratory functional responses can also be used for establishing quality control standards in mass-rearing projects (Allahyari et al., 2004). The development time within younger hosts and superparasitism are two crucial factors that should be considered in the parasitoid mass-rearing protocol and augmentative field release program to ensure production efficiency and prevent wastage of parasitoid reproductive potential. However, because behavioral differences between the laboratory and natural ecosystem can occur, field experiments are needed to further confirm the parasitoid–host interactions as observed in this study.

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